Standard Operating Procedure for Benthic Invertebrate Field Sampling

LG406

Revision 07, March 2002

TABLE OF CONTENTS

Standard Operating Procedure for Benthic Invertebrate Field Sampling Procedure

1.0 SCOPE AND APPLICATION

1.1 This standard operating procedure describes a method for collection and preservation of benthic invertebrate and sediment characterization samples from the soft sediment typical of Great Lakes deepwater benthic habitats.

2.0 SUMMARY OF METHOD

2.1 Three separate samples of benthic invertebrates are taken with a Ponar grab sampler at each designated sample site in each lake. Each sample is processed, preserved and stored separately. Elutriation may be used when the samples contain large amounts of sand, zebra mussel shells, or other debris that prevent sediment from quickly passing through a 500-µm mesh. During the summer cruise, a fourth Ponar sample is taken for grain size and chemical analysis.

2.2 During the spring cruise, samples are taken at five sites (Saginaw Bay, Green Bay, and the Western Basin of Lake Erie) and are analyzed specifically for the mayfly *Hexagenia*. During the summer cruise, all stations are sampled and analyzed for all benthic invertebrates.

3.0 EQUIPMENT

2 Full-size Ponar grab samplers
Sturdy tub to hold Ponar sample (2 to 4)
Elutriator, 500-µm mesh sleeve and deck stand
500-µm mesh sieve bucket
Hose/pump for water supply
Adjustable spray nozzle for hose
Funnel
1-L plastic field sample bottles (4 per site)
500-mL plastic field sample bottles (1 per site)
Labels and marking pens
Benthos field sheet

4.0 REAGENTS

4.1 37 % Formaldehyde

4.2 Rose Bengal stain (powder)

4.3 Reagent Preparation

Add approximately 1 g Rose Bengal stain per 20 L of 37% formaldehyde. This can be done on the ship, prior to arrival at the first site.

5.0 ON-STATION PONAR SAMPLER PROCEDURES

5.1 Sample Collection

5.1.1 Cock the arms of the Ponar grab sampler to open position and insert the spring loaded safety pin.

5.1.2 Lower the Ponar to the sediment surface. The grab sampler should be lowered slowly to within 5 m of the bottom, then allowed to free fall to the bottom. The jaws will close automatically as the grab sampler is raised from the sediment surface. If the Ponar grab sampler descends too quickly, it creates a "bow wave" that can push animals out from under the sampler, as well as strike the bottom at an improper angle. However, if it strikes the sediment without enough force, it will not penetrate deep enough for a good sample. Stopping the sampler during the descent may cause the trigger to release. The speed at which it is raised is not as critical as the speed of descent.

5.1.3 Once the grab sampler is on deck, check to see that the jaws are closed properly and that there is an adequate sample of sediment inside the Ponar.

5.1.4 Empty the grab sampler into a plastic tub.

5.1.5 Rinse the sediment and animals from the top screen and the interior of the Ponar.

5.1.6 If the substrate at the site is hard packed clay, sand or bedrock, the grab sampler will come up empty. Because the sample sites were originally located in depositional areas, none of these situations should occur. If these problems do occur, the station should be relocated to deeper water (moving as short a distance as possible, less than 500 feet) and the procedure re-started. If problems persist, consult with the GLNPO Chief Scientist on duty. Any decision to discontinue sampling *must be made by the GLNPO Chief Scientist*, and noted as such in the benthos field collection worksheet. If it is not possible to obtain a sample from the site, it might be necessary to revise the station location before the next cruise.

5.1.7 If the sediments are fine enough to quickly wash through the mesh, then use the sieve procedure (Step 5.2). If the sediment contains rocks, zebra mussel shells, or other debris and is too coarse to pass through the mesh easily, it will be necessary to elutriate the sample (Step 5.3).

5.2 Rinsing the Sample

5.2.1 Add water to the sample and mix gently with a spoon to break up lumps of sediment. Pour the sample slurry from the tub through a 500-µm sieve bucket which is placed over a second tub to catch the rinse water. Wash the sediment through the mesh with water at **VERY LOW PRESSURE.** Excessive pressure will result in damage to organisms, in particular oligochaetes, and will therefore compromise taxonomic analysis of the sample. Gently agitate the sieve bucket to aid in rinsing the fine sediment out of the sample. It may be necessary to sieve the slurry in small portions to prevent clogging of the mesh.

5.2.2 The sample should be rinsed until no more than 750 mL of material is left. In other words, the sample bottle should be less than ¾ full.

5.2.3 If there is too much material to reduce to 750 mL, then two sample bottles should be used. The fact that there are two bottles for the sample should be indicated on both the sample bottle labels, and the Benthos Field Collection Worksheet.

5.2.4 When rinsing is completed, concentrate the rinsed sample in one corner of the sieve bucket and wash it through a funnel into the sample collection bottle, and begin to process the next sample (Step 5.4).

5.3 Elutriating the Sample

5.3.1 Use the elutriation method only when the sample contains too much large material to wash quickly through a 500-µm mesh sieve. Visually examine the sediments during the elutriation process and note on the field sheet if there are large numbers of live or dead zebra mussels. If there are large numbers of shells, most likely at Lake Erie sites and near shore sites in the other lakes, the sample must be elutriated.

5.3.2 Place entire sample in the elutriator, fill it with water, and then gently stir the water and sediment together with your hand. This will suspend the animals and sediment in the elutriator. Agitating the water too vigorously will destroy a large number of animals (soft bodied oligochaetes are most susceptible) and compromise the laboratory results.

5.3.3 Stop stirring and let the sample stand for a few seconds. This allows the largest/heaviest sediments to settle and the animals to be poured off with the water.

5.3.4 Lift the handle edge of the elutriator and pour the water into the nozzle/net/field sample bottle GENTLY. This rinsing process should be repeated 8 times per sample. Release spent sediments over the side when finished.

5.3.5 The efficiency of the elutriator separation step will vary somewhat with the sediment type at the site. The number of times a sample is rinsed will affect the number of animals recovered (more rinses = more animals recovered). All biologists MUST use the same number of rinses during the entire cruise, regardless of sediment type encountered. This means that samples with high percentages of large detritus (e.g., samples from Lake Erie, Green Bay, or Saginaw Bay) may not separate well and two bottles per replicate may be needed to preserve the sample. Make sure both bottles are labeled the same and make a note how many bottles were used on the Benthos Field Collection Worksheet.

5.4 To complete replicate sampling, repeat Steps 5.1 through 5.3 twice more, for a total of three replicates at each benthic station visited.

5.5 Habitat Characteristics Sample

5.5.1 A fourth Ponar sample is taken for habitat characterizations. Sample collection follows steps 5.1.1 through 5.1.3.

5.5.1.1 Place the fourth sample GENTLY into a tub after allowing water to drain out of the Ponar. It is important not to disturb the sample.

5.5.1.2 Remove enough sediment from the top 2 - 3 cm of the sample to ⅔ fill a 500 mL bottle for organic content, then ⅔ fill a 1-L bottle with surface sediment for grain size analysis.

6.0 SAMPLE PRESERVATION AND LABELING

6.1 Sample Labeling

6.1.1 Field sample bottles should be labeled with lake, cruise, sample site, and parameter name (“benthos,” “nutrients” or “grain sz”). Duplicate and triplicate benthos samples are designated by a D and T, respectively, in the 7th field of the GLNPO number; all other samples have an S in that field. Labels are to be provided by GLNPO personnel one month prior to the start of each cruise.

6.2 Preservation of Biological Samples

6.2.1 Once the sample is elutriated or sieved, return the bottles to the ship board lab for preservation.

6.2.2 Add 50 to 100 mL of 37% formaldehyde with Rose Bengal to the sample. Top off the sample bottle with tap water. Invert 3 times. The final concentration will be 5 - 10% formaldehyde required for proper fixation and preservation. The higher concentration of formaldehyde should be used if there is more than 500 mL of organic material in the concentrated sample.

6.2.3 Wrap the top of the jar in parafilm to prevent leakage and store the sample in a designated cooler in the walk-in refrigerator.

6.3 Preservation of Sediment Samples

6.3.1 Samples for chemical and grain-size analysis should be stored in the shipboard freezer.

6.4 Benthos Field Documentation

6.4.1 Notes should be made in the field log book to indicate any changes to the normal sampling procedure (e.g., more than one bottle used; sample skipped by authority of GLNPO Chief Scientist; unusual substrate encountered, etc.).

6.4.2 The field technician should also complete the Ponar Grab Data sheet and enter the data into the onboard computer database.

7.0 QUALITY CONTROL

7.1 Precision of the sampling process is obtained by having all crew members follow the same steps in the same order for the entire cruise.

7.2 New crew members or those who have not previously performed the procedures must read a copy of this SOP before boarding the boat. A copy will also be available on the boat.

7.3 The more experienced benthic sampler must closely supervise the new sampler for at least two stations after which the new sampler is expected to perform sampling unsupervised.

8.0 SAFETY AND WASTE HANDLING

8.1 Refer to GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys.

8.2 All applicable safety and waste handling rules are to be followed. These include proper labeling and disposal of chemical wastes. Over-board discharges of chemical wastes are forbidden.

8.3 During sampling, caution, common sense, and good judgment should dictate appropriate safety gear to be worn in any given situation on deck. Hard hats, gloves, and steel-toed shoes must be worn in working conditions where there is a possibility of injury to the head, hands, or feet; however, if in doubt, please ask the Chemical Hygiene Officer.

8.4 Collecting samples in cold weather, especially around cold water bodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather. For specific

information regarding sampling during cold conditions, please refer to the US EPA GLNPO *Standard Operating Procedures for Winter Operations* (December 1994, or as amended).

8.5 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.

8.6 Work vests must be worn while working on the fantail and Rosette deck.

8.7 Safety glasses, hard hat and gloves are to be worn as directed by the ship-board chemical hygiene officer. The 37% formaldehyde solution will be stored in the hazardous chemical storage locker (enough formaldehyde for a shift's stations can be kept in the hood in the biology lab) and used under a chemical hood.